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Tracing a bottleneck: comparative phylochronology of *C. sociabilis* and *C. haigi* through 10,000 years

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Introduction

Phylochronology – population genetics through space and time.

Understanding how animal populations respond to climatic change is an important conservation question. Particularly important is the understanding of the dynamics and persistence of endemic species over long temporal scales. We studied the phylochronology of two species of *Ctenomys* (tuco-tucos) in Patagonia using ancient DNA. By combining the fossil record with molecular techniques, we monitored changes in abundance concurrent with changes in genetic diversity over a timescale of thousands of years.

C. sociabilis

- endemic to area approx. 1400 km²
- modern microsatellite data suggests recent population bottleneck (Lacey 2001)
- 1,000 years in Estancia Nahuel Huapi (N = 14) (Hadly *et al.* 2003) and modern studies (Lacey pers. comm.) have found no variation at *cyt-b*
- social tuco tuco, females philopatric

C. haigi

- larger geographic range
- no modern evidence of bottleneck
- solitary behavior

Figure 1. Vegetation map of area near cave sites **Estancia Nahuel Huapi** and **Cueva Trafal** (adapted from Markgraf 1983). *C. sociabilis* is endemic to the area outlined in red, and edge of the range of *C. haigi* is in blue.

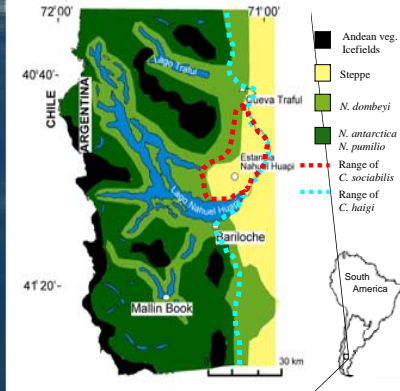
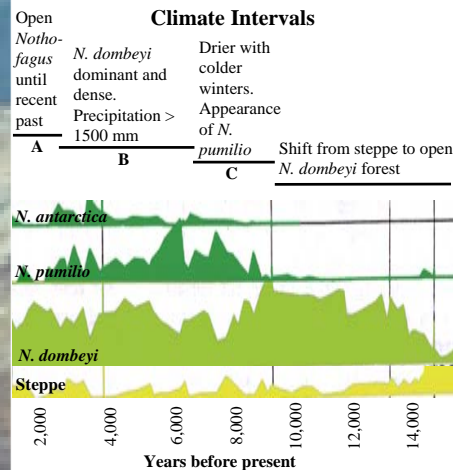


Figure 2. Pollen record from Mallin Book (see map) shows late-Holocene changes in habitat and climate (Markgraf 1983). *Ctenomys* prefer open steppe habitats (yellow) which have declined over the last 14,000 years.



Objectives

- Obtain ancient DNA from fossils up to 10,000 years old contained in Cueva Trafal, to compare with 1,000 years of no variation at *cyt-b* in Estancia Nahuel Huapi
- Relate changes in diversity and abundance to changes in climate

Methods



45 teeth extracted (protocol: Hadly *et al.* 2003)
8 stratigraphic units (3 climatic intervals dating from 10,000 years ago to present)
230-365 bp cytochrome-*b* amplified in 3 fragments.

- Stringent contamination controls.
- Primers designed for Octodontid spp.
- Spatial and temporal separation of DNA and PCR products.
- No prior use in the lab of *Ctenomys* modern tissue or DNA.

Results

Ancient DNA

C. sociabilis - Diversity!

- 5 haplotypes, 13 variable sites
- 2 historic haplogroups, 2.5 % seq. divergence
- 1 modern haplotype, 0 % seq. divergence

C. haigi

- 5 haplotypes, 5 variable sites
- 1 historic haplogroup, 1.4% seq. divergence
- 2 modern haplogroups, 2.2% seq. divergence

Figure 3. Although the abundance of *Ctenomys* fossils (NISP = 558) remains constant through all levels in Cueva Trafal (Pearson and Pearson 1993) the relative frequency, as identified based on ancient DNA in Cueva Trafal, showed a decline in *C. sociabilis* from 80% to 30% of the sample from 8,000 years ago to the present (sample size shown)

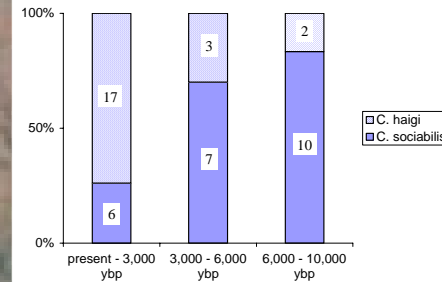
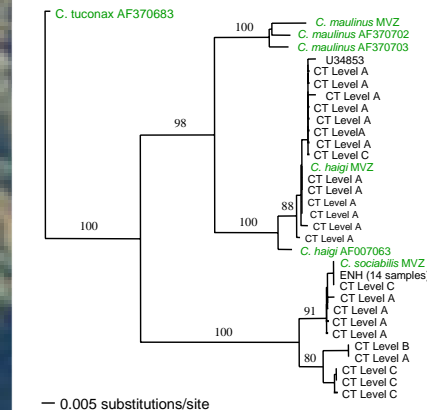
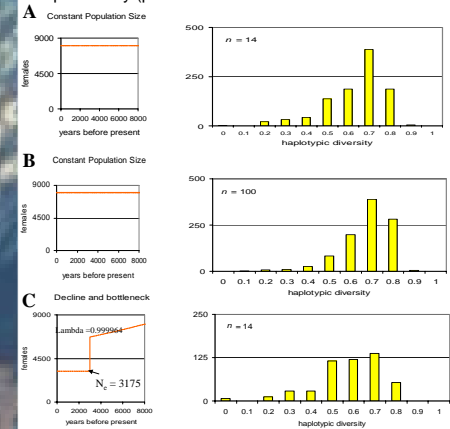


Figure 4. Neighbor-joining tree of ancient (in black) and modern (in green) *Ctenomys* samples based on 365 bp.



Simulation

Figure 5. Drift and mutation were simulated for a closed, non-structured population representing the species range with initial $N_e = 8042$ for 8,000 years to investigate probability of zero variation. Haplotype diversity was calculated based on n haplotypes randomly sampled from time period 1,000 y.b.p. for the following models: (A) null model: $n = 14$ to represent realistic sampling in Estancia Nahuel Huapi. (B) null model: $n = 100$ to investigate effects of sampling. (C) Realistic population decline followed by a bottleneck ($n = 14$) resulted in a low probability ($p=0.014$) of zero haplotypic diversity.



Conclusions

- Despite lack of modern variation, previous variation existed
- Long-term decline of *C. sociabilis* apparent from Cueva Trafal
- Simplistic models of the population demography cannot explain observed lack of diversity. Further research will involve coalescent modeling of more complex hypotheses, and estimation of effective population size, and severity and timing of bottleneck, as well as coalescent-based models involving genetic structure.

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